

THE EFFECTS OF CONJUGATION WITHIN A CLONE OF
PARAMECIUM AURELIA

DANIEL RAFFEL

A Dissertation Submitted to the Board of University Studies of the Johns Hopkins
University in Conformity with the Requirements for
the Degree of Doctor of Philosophy

Baltimore
February, 1930



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THE EFFECT OF CONJUGATION WITHIN A CLONE OF PARAMECIUM AURELIA

DANIEL RAFFEL

(From the Zoölogical Laboratory of the Johns Hopkins University)

INTRODUCTION

On the effects of conjugation in paramecium, particularly in relation to the production of inherited variations, the results of investigators are in conflict. Jennings (1913), working with both *Paramecium aurelia* and *Paramecium caudatum*, reported that conjugation increased inherited variations: that it caused the production of diverse biotypes. The members of a clone—a population derived by fission from a single individual, whether an ex-conjugant or not—remained nearly or quite uniform in their inherited characteristics so long as conjugation did not occur among them. But after conjugation within such a clone, the inherited characteristics of descendants of the different ex-conjugants had become diverse. Thus by conjugation many different biotypes had been produced, the descendants of each ex-conjugant constituting a single uniform biotype.

Calkins and Gregory (1913), on the other hand, reported that there is in *Paramecium caudatum* as much variation among the descendants of the four individuals produced by the first two fissions of a single ex-conjugant as was found between the progeny of different ex-conjugants. They conclude that, "The results of this study show that physiological and morphological variations in the progeny of a single ex-conjugant of *Paramecium caudatum* are fully as extensive as the variation between the progenies from different ex-conjugants" (p. 523).

Jennings (1916, p. 528, and 1929, p. 188) has tried to show that the results of Calkins and Gregory are invalidated by uncontrolled sources of error. On the one hand, he holds that their method of culture permitted continuing environmental differences between their different populations, such as would give rise to differences that would appear to be hereditary, although they were not. On the other hand, he notes the occurrence of conjugation within some of their cultures and the fact that this might readily have occurred undetected. This would vitiate their conclusions.

Obviously, the situation calls for a new investigation of the matter, in which such methods shall be employed as shall certainly exclude the possibility that environmental differences affect the results, while at the same time the occurrence of unobserved conjugation is excluded. It is such an investigation that is here presented. In order to assure a uniform environment for all the lines of descent an elaborate technique was employed. This is described on later pages. The method involved, first, the use of a synthetic culture medium of known composition, with pure cultures of food organisms and uniform glassware; second, continuation of the uniform conditions by the cultivation of the paramecia under aseptic conditions; third, frequent testing of the culture fluid in which the organisms have lived in order to ascertain whether the uniformity of the environment has been maintained. In addition, the organisms are cultured singly and transferred daily to new drops of culture fluid, so that it is impossible for conjugation to occur. Continuing diversities between lines cultivated simultaneously under such conditions can be interpreted only as caused by constitutional differences among the organisms, not as due to diversities in food or cultural conditions, or other extrinsic factors.

Taking these precautions, two comparisons are made. First, a population descended from different ex-conjugants is compared with a population derived by fission from non-conjugants of the parent clone. Second, four lines descended from each ex-conjugant are compared with one another, and the several such different clones are similarly compared. In this way it is possible to determine whether increased hereditary variation and differentiation into diverse biotypes are produced by conjugation.

The investigation was suggested to me by Professor H. S. Jennings, and my thanks are due to him for assistance throughout the work. I am also indebted to Rose Mahr Raffel, who assisted in the carrying out of the experiment, and without whose aid cultures of this magnitude, using the elaborate technique here employed, could not have been carried through.

MATERIALS AND METHODS

In this investigation an elaborate technique was used in order to subject all of the lines to identical environmental conditions. Great care was taken to eliminate any possible sources of variation. To this end the culture fluid, the food supply and the glassware used were standardized to as great an extent as was possible. The work which has been carried on for several years by Hartmann and his associates

at the Kaiser-Wilhelm Institut für Biologie has made possible the use of synthetic culture fluids and pure cultures of food organisms for the cultivation of protozoa. The use of pure cultures of unicellular algæ as food organisms appears first in the work of Luntz (1926) on the rotifer *Pterodina elliptica* and more recently in the work of Adolph (1929) with the ciliate *Colpoda*. The results of the work of Hartmann and his associates are given in a recent paper of Belar (1928). The following pages contain a detailed account of the methods used to obtain uniformity in the environmental conditions throughout this experiment.

1. Culture Fluid

The culture medium used was a physiological salt solution of known composition. After many attempts to find a solution in which the race of *Paramecium aurelia* which was used would live, it was found that if the solution described by Pringsheim (1928) for the cultivation of algæ was altered so as to be neutral, it furnished an excellent medium for this organism. This modification was obtained by replacing the KH_2PO_4 used by Pringsheim by an equal molar concentration of K_2HPO_4 . The composition of the solution was KNO_3 0.5 gram, K_2HPO_4 0.06 gram, MgSO_4 0.02 gram, FeCl_3 0.001 gram, water 1000 grams. The water used in making this solution was redistilled from a still made of Pyrex glass and had in all cases a conductivity less than 1.05×10^{-6} mho. This solution was made up in quantities of one liter. It was then divided into portions of approximately 15 cc. in test tubes. These test tubes were plugged with non-absorbent cotton and the solution was sterilized in the autoclave for 15 minutes under 15 pounds of steam pressure. The solution was kept in this way for periods varying from a few days to two months before it was used. Tubes tested at intervals showed no bacteria and no measurable alterations in composition.

2. Food Organism

The food organism used was a unicellular green alga, *Stichococcus bacillaris*.¹ This was cultivated on 0.05 per cent Benecke's agar composed of water, 1000 grams; Agar-Agar, 15 grams; NH_4NO_3 , 0.2 gram; CaCl_2 , 0.1 gram; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1 gram; and K_2HPO_4 , 0.1 gram. The components of this agar were boiled together until the agar-agar was all dissolved. Five cc. portions were poured into test-tubes which were then sterilized in the autoclave under 15 pounds of steam pressure for fifteen minutes. These tubes were then "slanted"

¹ I am indebted to Professor W. R. Taylor of the University of Pennsylvania for the identification of this organism.

in order to obtain a large, easily accessible surface. Twenty of these tubes were seeded from a pure culture of the alga on successive days. After this the slants were used in the order in which they had been seeded and as they were used they were replaced by new tubes seeded from them. The tubes in which the alga was cultivated were kept constantly before a north window in order to obtain sufficient light.

Each day the tube of *Stichococcus* to be used that day and a fresh tube of the culture fluid were opened close to a flame into which their open ends were immediately thrust. Then a small quantity, approximately 5 cm., was scraped from the agar with a platinum loop which had just been sterilized in the flame. This small quantity of the alga was then quickly suspended in the solution and both tubes were immediately restoppered. Then a new tube of agar was seeded from the same tube and replaced in its proper place in the rack. Many tests of the suspension were made from time to time and in no case was any bacterial contamination found. An effort was made to have the suspension of alga in the solution always of the same density. However, no method more accurate than a comparison of the appearance of the tubes was found for determining the success of this effort. For this reason, preliminary experiments were performed in order to determine whether or not the quantity of algæ used affected the rate of reproduction of the paramecia. It was found that sufficient algæ to produce a slight greenish tinge in the suspension furnished enough food for these organisms. Greater densities than this had no effect on the rate of reproduction even when they were far in excess of any used in the actual experimental work. At all times an excess of algæ was assured and the drops containing the paramecia always showed a large number of the algæ at the end of the period during which the organisms remained in them.

It was found, however, that if the paramecia were kept in this solution with this single food organism, they were unable to live and reproduce. If a very slight trace of a *Bacillus candicans* was present, this difficulty was eliminated.² Attempts were made to cultivate the paramecia on this bacillus in the absence of the alga. All such attempts failed, and when a mixture of the two food organisms was used, the food vacuoles were dark green in color—indicating that the food supply was composed mainly of the alga. After a slight trace of this bacterium was once introduced into a culture of paramecium, it was perpetuated in the transfers of the organisms. As far as it was possible to de-

²I am indebted to Professor W. W. Ford, Professor of Bacteriology in the School of Hygiene and Public Health of the Johns Hopkins University, for the identification of this bacillus.

termine by plating in the usual way, this bacterium was present in approximately the same quantity from day to day in all of the many cases tested at random. It was thought advisable, however, to determine whether or not differences in the quantity of this organism present affected the rate of fission of the paramecia. There was no difference in the effect produced by the presence of any quantity of the bacterium less than that required to make the drops of culture fluid appear milky. At no time during the course of this investigation was this condition approached.

3. *Glassware*

The various lines of paramecium used in this investigation were cultivated on slides with two concavities. It was found from preliminary work that different slides affected the paramecia differently. On some slides representatives of all the lines tested reproduced more rapidly than did other representatives of the same lines on other slides. The pH of drops of culture fluid which had remained on the different slides was tested and was found to vary greatly. Drops of fluid which had been identical when placed on the slides were found to vary by a whole pH unit within twenty-four hours. This showed that the glass of the various slides differs in solubility. New slides were then obtained, all of the same kind of glass. These slides were of French origin. After two days the organisms grown on these slides died out. No amount of washing the slides with various kinds of solvents made it possible to cultivate organisms on them. Investigation disclosed that this French glass is made by a process involving the use of lead. It appears that the presence of this element was responsible for the toxic effects of these slides on the organisms. When this was discovered, new slides were obtained which were of white glass and were all produced by the same manufacturer. These slides were the only ones used in this investigation. Before they were used they were thoroughly washed in running water. Then they were washed in ether and 95 per cent alcohol in order to remove any organic matter with which they might have been contaminated. They were again thoroughly washed with running water, rinsed in several changes of tap water and finally rinsed in hot distilled water. Each day the slides were thoroughly washed in the following manner. First they were held, individually, in running tap water and the depressions were rubbed well with the thumb. They were then placed in a receptacle containing clean tap water. In this receptacle they were rinsed three times. Then, after the last tap water was thoroughly drained off, the slides were covered with hot distilled water. They were then dried on racks.

In order to prevent contamination of the cultures by bacteria in the air, Petri dishes 100 mm. in diameter and 15 mm. deep were used as moist chambers. This made it possible to transfer the organisms with a minimum of exposure to the air. The dishes contained water at the bottom; the two slides to each dish were supported above this on strips of glass. After the Petri dishes, the slides, and the glass plates were assembled, they were heated in the hot air sterilizer for one hour at 150° C. In order to facilitate the handling of the numerous dishes which were used, baskets were made from $\frac{1}{4}$ inch wire netting which held a dozen Petri dishes in four tiers of three dishes each.

The organisms were transferred by means of capillary pipettes. Each of these contained a plug of cotton inserted into its wide end. This is a precaution necessary to prevent contamination of the cultures by microorganisms which would otherwise be introduced by the rubber bulbs used on the ends of the pipettes. The glass part of the pipettes with their cotton plugs were kept in large museum jars, in which they were heated in the hot air sterilizer for one hour at 150° C. before each time they were used.

4. Method of Transferring Organisms

Before the daily transfers were made, the Petri dishes were removed from the hot air sterilizer. Then two drops of the culture suspension were dropped into each concavity. Large pipettes which were drawn out until the ends were 2 mm. in diameter were used for this purpose. These pipettes, like the ones used for transferring the paramecia, were protected by cotton plugs and were sterilized before each time that they were used. The mouth of the test tube containing the suspension of culture fluid was sterilized in the Bunsen flame each time that it was opened. The tops of the successive Petri dishes were then raised on one side, the pipette was introduced and two drops were allowed to fall into each concavity. Four dozen dishes were prepared in this manner at one time. From time to time bacteriological plates were prepared from culture medium which was treated in the manner described above, after it was left for twenty-four hours. In every case the plates were negative, thus indicating that the technique was absolutely dependable.

In transferring the animals a Petri dish containing the two slides was placed on the stage of the binocular microscope. Another dish containing new culture fluid was placed at the experimenter's right. One organism was then taken from each concavity and transferred to the corresponding concavity of the new dish. This was done very rapidly, using a clean pipette that had just been removed from the jar of

sterile pipettes. A separate pipette was used for the organisms of each dish. The new dishes were then removed to the constant temperature chamber, in which they were left at a temperature of approximately 24° C. (There was in the history of the cultures variation in temperature from 22.2°–26.2° C.)

5. *Isolation and Sterilization of the Clone*

The various lines of *Paramecium aurelia* used in this investigation are the descendants of a single individual which was isolated from a mass culture in the laboratory on July 29, 1929.

Parpart (1928) has shown that spores of bacteria may be, and often are, carried within paramecium and that in washing these organisms, precaution must be taken to eliminate these spores as well as the bacteria external to the paramecium. For this reason, when the individual which was used to start the clone for this investigation was washed, the precautions suggested by Parpart were observed. The individual was first washed successively in five concavities containing sterile culture fluid. Then at intervals of one hour it was washed through five more similar quantities of fresh culture fluid. It was then placed in a concavity containing the regular culture suspension described above in which there was a slight trace of the *Bacillus candicans*. No bacteria were added at any later time. From time to time throughout the course of the experiment bacteriological plates were made from drops from which the paramecia had been removed. Several dishes containing both ex-conjugant and non-conjugant lines were taken at random for this purpose. At no time did any plate made in this way indicate the presence of any bacteria except the bacterium which had been introduced at the beginning.

THE EXPERIMENT

1. *Plan*

The plan of the experiment was as follows: A clone was obtained by allowing a single individual of *Paramecium aurelia* to multiply. A portion of the clone was induced to conjugate, while another portion was kept without conjugation. The former, after the separation of the pairs, yields lines of descent that constitute the ex-conjugant population, the latter the non-conjugant population. These two populations are later compared as to their mortality, fission rate, variation and the inheritance of the variations.

For comparison with the results of Calkins and Gregory, a method similar to theirs was employed for the grouping and subdivision of

the ex-conjugant lines. Each of the two members of a pair was allowed after separation to divide twice, yielding four individuals of common origin, the four *quadrants*. From each quadrant a line of descent was obtained. Each set of four quadrants derived from a single ex-conjugant is called, for convenience, a *tetrad*. The variation within single tetrads is compared with the variation among lines belonging to different tetrads (and so derived from different ex-conjugants). This tests whether the diversity among the descendants of a single ex-conjugant is as great as that between those of different ex-conjugants (as is maintained by Calkins and Gregory).

2. Description

The experiment was begun with the isolation of a single organism on July 29, 1929. The progeny of this individual were propagated on slides by daily transfer until August 5, 1929. By this time there were approximately 1500 individuals present. On the morning of August 5, all of the individuals, except one, from each concavity, were transferred to two small sterile culture dishes contained within Petri dishes. No fresh culture fluid was added to those culture dishes and the least possible quantity was carried over with the organisms. The other organisms were transferred to clean slides in the usual manner. These latter ones were the source from which the non-conjugant lines used in this experiment were obtained. The process of transferring this number of animals occupied several hours. Before all the organisms had been transferred conjugation had begun in the two culture dishes. One hundred and twenty pairs of conjugants were removed from the culture dishes and numbered in order of their removal. The next morning the pairs had separated. The two members of each pair were transferred to the two concavities of a clean slide. The non-conjugants, one from each dish which had been transferred to slides on August 5, were transferred to clean slides until 112 non-conjugants had been transferred. The number of fissions was recorded in the case of the non-conjugants. On August 7-8 the ex-conjugants completed their first two divisions, giving rise to the four lines or quadrants from each of the ex-conjugants which were to be propagated in this experiment.

On August 7th and 8th the non-conjugant lines and the ex-conjugant lines were so distributed that no two non-conjugant lines or lines from the same tetrad were cultivated in the same Petri dish. This was done so that if any correlation was found between the quadrants of a tetrad or between lines of the non-conjugant population, it could not be the result of cultivation on the same slides or in the same dishes.

From August 6th to September 10th inclusive, each line was transferred each day (except on August 8th and 10th as described below). On August 8th and August 10th the amount of work was so great that it was not possible to complete the transferring until after midnight. The lines which were not transferred until after midnight on these days were not transferred again for approximately 36 hours. On August 11th all of the lines which were incomplete because of losses were discarded. When this was done, the number of lines retained was the maximum number that two persons could transfer once daily, using this involved technique. From August 11th to the close of the experiment on September 10th, all the lines surviving were transferred daily.

The actual numbers isolated at the beginning of the experiment were for the non-conjugants 112; for the ex-conjugants 405 lines or "quadrants" derived from 105 different ex-conjugants, belonging to 58 different pairs. The numbers were reduced by accident or death of lines, so that the actual numbers of lines available for comparison were, for the first ten days of the experiment, 66 non-conjugants, 324 ex-conjugants; for the first twenty days, 64 non-conjugants, 295 ex-conjugants; for the entire period of 36 days, 46 non-conjugants, 115 ex-conjugants.

During the first week following the beginning of the experiment a rather large number of deaths occurred among the non-conjugant lines. After this period there occurred a period of about three weeks during which deaths among the non-conjugant lines were rare. Many of the deaths which occurred during the early part of this period were lines that had stopped dividing during the earlier period. On the twenty-fifth day of the experiment (August 29th), the rate of mortality among the ex-conjugant lines increased rapidly. This was followed two days later by an increase in the rate of mortality among the non-conjugants. This high rate of mortality continued for nearly ten days. The occurrence of this high rate of mortality in the ex-conjugant lines beginning twenty-five days after conjugation was accompanied by a general depression in all the lines. This fact and the occurrence of two such periods in the non-conjugant lines, separated by a period of about twenty-five days, led to the suspicion that these were periods of endomixis. On September 6th many of the excess animals from the non-conjugant and ex-conjugant lines were stained and mounted for study. The individuals from the ex-conjugant lines showed in many cases the conditions of late stages of endomixis. Numerous fragments of macronuclei were present, and in one case the organism was found to be at the climax of the endomictic process. The representatives of the non-conjugant lines showed on the whole the conditions

typical of earlier stages of endomixis. Large irregular macronuclei were found, often accompanied by large fragments. It seems clear, therefore, that the periods of high mortality were periods of endomixis; a relation which other investigators have observed.

On September 10th, thirty-six days after conjugation had occurred, the experiment was discontinued. At this time 46 lines of non-conjugants and 115 lines of ex-conjugants were still in existence.

3. Results

The experiment was designed to supply data mainly upon the rate of reproduction, its variability and the inheritance of the variations, in the ex-conjugants and non-conjugants. It yields also certain data on comparative mortality, which will be given first.

A. Mortality

A considerable number of the lines of both the non-conjugants and ex-conjugants died out during the thirty-six days of culture. The percentages surviving in each group at certain periods after the beginning of the experiment, are the following:

After	20 days	25 days	35 days
Non-conjugant lines	73.0	68.6	51.7
Ex-conjugant lines	79.2	67.9	30.8

Thus on the whole the mortality is much greater among the lines descended from the ex-conjugants. At the end 51.7 per cent of the non-conjugant lines were alive as against 30.8 per cent of the ex-conjugant lines.

B. Rate of Reproduction

The basic data as to comparative rate of reproduction in the non-conjugants and ex-conjugants are given in Table I. The number of fissions for both groups is reckoned from August 7th, on which day all of the ex-conjugants divided once or twice. Thus the statistics are not affected by any delay in fission due to the process of conjugation itself.

Table I shows at *A* the number of fissions for the different lines for the 20 days of culture beginning August 7th and ending August 26th; throughout this period there were 64 lines of non-conjugants and 295 lines of ex-conjugants, the latter derived from 99 different ex-conjugants and so forming 99 tetrads. At *B* are shown the distribution

TABLE I

Distributions of the total numbers of fissions and the daily fission rates, for the different lines cultivated, for different periods of the experiment. The mean fissions and fission rates are also given (for certain periods), for the different tetrads (total lines derived from a single ex-conjugant).

A. Fissions for the First 20 Days, Aug. 7 to 26 inclusive																									
		Number of Fissions in 20 Days																				Total Lines	Mean Fissions		
		16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35			36	37
Non-conjugants.....														2	1	5	2	17	12	14	6	4	1	64	32.9 ± 0.2
Ex-conjugants.....		1		1		1	1	1	2	3	5	7	12	27	30	55	43	45	36	14	10	1	1	295	31.3 ± 0.1
Means of tetrads.....		1	1			1	1			1	1	2	3	5	15	17	20	19	11	3			99	31.2 ± 0.2	

B. Fissions for the 35 Days, Aug. 7 to Sept. 10 inclusive																											
		Number of Fissions in 35 Days																				Total Lines	Mean Fissions				
		38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57			58	59	60	61
Non-conjugants.....											1	1			4	1	2	2	4	5	10	6	5	3	2	46	56.1 ± 0.3
Ex-conjugants.....		2	1	1	1	1	1	1	2	3	2	2	5	9	5	13	18	16	9	6	7	6	3	2	115	53.3 ± 0.3	

Mean Daily Fission Rates, for the Period Which Each Line Lived, for All Lines That Lived More Than 10 Days

		Mean Daily Fissions																				Total Lines	Mean Daily Rate			
		.85	.90	.95	1.00	1.05	1.10	1.15	1.20	1.25	1.30	1.35	1.40	1.45	1.50	1.55	1.60	1.65	1.70	1.75	1.80			1.85		
Non-conjugants.....																									66	1.58±0.01
Ex-conjugants.....		1	2		1	1	2	4	7	15	18	20	24	36	50	71	29	24	14	4			1	324	1.48±0.01	
Tetrads.....			1			1	1		1	3	1	6	11	22	19	16	12	7						101	1.47±0.01	

of the numbers of fissions for the 46 surviving non-conjugant and 115 ex-conjugant lines that survived throughout the entire period of 36 days, August 6th to September 10th. At *C* in Table I are shown the mean fission rates for the total period of survival, for all the lines that lived more than 10 days.

Mean Rate.—As Table I shows, the mean rate of reproduction for the non-conjugant and conjugant groups did not differ greatly, although in every case the mean rate for the non-conjugants is higher by a small but significant amount. The mean fission rate for all non-conjugant lines is 1.58 ± 0.01 ; for all ex-conjugant lines, 1.48 ± 0.01 .

C. Variation in Fission Rate

But it is in the variation of the fission rate that the difference between the non-conjugants and ex-conjugants is striking. An inspection of Table I shows at once that the variation in the ex-conjugant lines is much greater than that in the non-conjugant lines. For the first twenty days, the number of fissions in the non-conjugant lines varies from 28 to 37, a range of 10. For the ex-conjugant lines, in the same period the range is from 16 to 37, a range of 22, more than double that for the non-conjugants. For the entire 35 days, the non-conjugant lines range from 47 to 61, the ex-conjugant lines from 38 to 61. The mean daily fission rates (*C*, Table I) vary in the non-conjugants from 1.25 to 1.75; in the ex-conjugants from 0.85 to 1.85. The range for the former is 0.55; for the latter 1.05, or nearly double that for the non-conjugants. The fission rate for the lowest lines of ex-conjugants is far below that for the lowest non-conjugants, and the highest ex-conjugant line is above the highest non-conjugant. Conjugation within the clone has caused a wide spreading out of the fission rates; it has produced stocks with lower, and with higher, rates than any found in the clone before it has conjugated.

Computation of the standard deviations and coefficients of variation shows the same great increase in variation as a consequence of conjugation. The means, standard deviations and coefficients of variation, computed from the data shown in Table I, are given in Table II.

As Table II shows, the coefficient of variation of the ex-conjugant lines is for the first 20 days 158 per cent of that of the non-conjugants; for the entire 35 days it is 187 per cent of that of the non-conjugants. For the mean daily fission rates of the different lines, the coefficient of variation for the ex-conjugants (10.14) is 139 per cent of that of the non-conjugants (7.28).

The comparative distribution of the fission rates of non-conjugants and ex-conjugants, as shown in Table I, are worthy of notice. In the first 20 days (*A*, Table I) 22 lines of ex-conjugants, or 7.4 per cent of all, show fewer fissions than any of the non-conjugants. In the entire 35 days (*B*, Table I), the proportion is nearly the same: 7.8 per cent of the ex-conjugant lines lie below all of the non-conjugant lines. At the opposite extreme the two sets are alike; the highest lines have the same number of fissions in the two cases. In mean daily fission rates, 18 lines of ex-conjugants, or 5.6 per cent of all, lie below all of the non-conjugant lines, while one ex-conjugant line lies above all the non-conjugant lines.

TABLE II

Means, standard deviations and coefficients of variation of non-conjugant and ex-conjugant lines, for the numbers of fissions during certain periods; and for the mean daily fission rates of the different lines. Based on the data given in Table I.

A. Numbers of Fissions						
	First 20 Days			Total 35 Days		
	Mean	Stan. Dev.	Coef. Var.	Mean	Stan. Dev.	Coef. Var.
Non-conjug'ts	32.9±0.2	1.88±0.11	5.70±0.34	56.1±0.3	3.19±0.22	5.68±0.40
Ex-conjugants	31.3±0.1	2.83±0.08	9.02±0.25	53.3±0.3	4.49±0.20	8.42±0.38

B. Mean Daily Fission Rates of the Different Lines			
	Mean	Stan. Dev.	Coef. Var.
Non-conjugants...	1.58 ± 0.01	0.12 ± 0.01	7.28 ± 0.43
Ex-conjugants...	1.48 ± 0.01	0.15 ± 0.00	10.14 ± 0.27

It is clear, therefore, that conjugation within the clone has much increased the variability of the fission rate, and that one of the factors in the increased variability is the production of a considerable number of ex-conjugant lines that have a lower fission rate than any lines among the non-conjugants.

D. Variation among Quadrants Derived from a Single Ex-conjugant, Compared with Variation Between Lines Derived from Different Ex-conjugants

Calkins and Gregory (1913) reached the conclusion that the variation between different quadrants (the four lines derived from a single ex-conjugant) was as great as that between lines derived from diverse ex-conjugants. Lines derived from a single ex-conjugant constitute,

of course, a clone within which conjugation has not occurred; so that according to this result, there is no increase of variation in consequence of conjugation within the clone. To test this particular matter, the variation between the different quadrants of the same tetrads (each tetrad derived from a single ex-conjugant) was compared with the variation among progeny of the different ex-conjugants. For each tetrad records of only two to four lines are available, so that the coefficients of variation within the tetrad are not statistically adequate, but the general result is of interest. For the number of fissions during the first 20 days of the experiment, the mean coefficient of variation for the lines constituting a single tetrad was 4.53; for the means of the diverse tetrads (progeny of the diverse ex-conjugants), the coefficient of variation was 8.32. For the average daily fission rate, the mean coefficient of variation for the lines constituting a single tetrad was 5.22; for the diverse tetrads it was 8.57.

As will be seen by comparison with Table II, the mean variation within tetrads (4.53) is of a similar order to the mean variation for non-conjugant lines of a clone (5.70) (that is, to the variation within a clone in which conjugation has not occurred). On the other hand, the variation when the different tetrads are compared (8.32) is much greater, and is similar to the variation (9.02) when all the lines derived from ex-conjugants are compared. This indicates strongly that the four quadrants produced by the first two divisions of an ex-conjugant do not differ in any general way from any other products of fission of a single individual. Further, the similarity between the coefficients of variation for all ex-conjugant lines taken separately, and that for the means of the diverse tetrads, indicates that the variation among the ex-conjugant lines is due mainly to the inherent differences between the ex-conjugants.

The higher variation among diverse tetrads, as compared with less variation between the quadrants belonging to the same tetrads, may be further shown by comparing the maximum differences found (1) between any two lines of the original non-conjugant population; (2) between quadrants belonging to a single tetrad; (3) between the means of diverse tetrads; and (4) between any two ex-conjugant lines. These comparisons are given in Table III.

This table shows that the maximum difference within any of the 99 tetrads and the maximum difference between any two non-conjugant lines of the original population are very nearly identical. On the other hand, the maximum differences between any two ex-conjugant lines are only slightly greater than the maximum differences between

any two of the tetrads. (It is to be expected that the maximum differences between two tetrads would be slightly smaller than that between the two ex-conjugant lines which differ most, since the fissions for tetrads are usually the means of two to four lines.) Thus the single tetrads do not significantly differ in their variability from the general non-conjugant population, while the variation between the different tetrads is much greater than that within the tetrads.

TABLE III

Maximum Differences Between Lines Having Different Relations to Each Other with Respect to Conjugation

	Total Fissions First 20 Days	Average Daily Fission Rate
Maximum difference between two non-conjugant lines of the original population . . .	9	0.50
Maximum difference within any tetrad . . .	10	0.58
Maximum difference between two means of tetrads	17.75	0.84
Maximum difference between two ex-conjugant lines	21	1.00

The matter may be tested further by determining whether there is correlation in fission rates between the members of the tetrads. If the different quadrants within the tetrads differ as much as do the members of different tetrads, there should be no correlation between the members of the tetrads. If, on the other hand, the different quadrants of the single tetrads show a significant correlation, this will demonstrate that such quadrants are not so unlike as are different lines of the ex-conjugant population taken at random.

The data for this comparison are shown in Table IV, based on the numbers of fissions during the first 20 days of the experiment. The fissions for each quadrant of each tetrad are entered as X against the fissions for each other quadrant of that same tetrad as Y. As some of the tetrads had but two surviving lines, others three or four, the total number of entries in the table is 332 pairs. Since the members of the tetrads are like variates, the correlation must be computed as for a symmetrical table in which each pair is entered twice, in reverse order (see Jennings, 1911, for the method of computation).

The coefficient of correlation between the fission rates of the quadrants of the same tetrad, obtained from this table, is very high, amounting to 0.854 ± 0.007 . Beyond question, therefore, the quadrants derived from a single ex-conjugant are much more alike in their fission rates than quadrants derived from diverse ex-conjugants.

All the four lines of evidence thus agree in showing clearly that a population composed of different ex-conjugants of a clone has a higher variation in fission rate than do the offspring of single ex-conjugants. (1) The coefficient of variation is much higher for the ex-conjugant population than for the non-conjugant. (2) The coefficients of variation for quadrants belonging to single tetrads is much less than the coefficient of variation for the means of diverse tetrads. (3) The maximum differences between lines within tetrads are much less than the maximum differences between means of different tetrads. (4) There is a very high correlation (0.854) between the lines or quadrants derived from the same ex-conjugant. These four lines of evidence establish firmly the fact that conjugation within a clone causes increase of variation.

TABLE IV

Paramecium aurelia. Correlation between total number of fissions, August 7-26, of each member of the tetrad with every other member.

	18	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	
16	1																	1
22			1							1								2
23										1								1
24		1	1							1								3
25						2												2
26						1				1								2
27					1	3				2	1							7
28				2	2			1	1	3	2	1				1		13
29						1		1	2	3	6	7	3	2	3			28
30					1	2			6	6	8	6	3	1	1	1		35
31					1		4	2	5	7	14	5	5	7	3	4		57
32					1	1		4	5	6	7	7	10	5	2	1		49
33							1	1	6	8	10	11	10	10		1		58
34							1		3	1	9	3	16	6	3	1		43
35											3	7	3	4	1		1	19
36						1	1	1		1	1			4		1		10
37												1			1			2
	1	1	2	2	6	11	7	10	28	41	61	48	50	39	14	10	1	332
	$r = + 0.854 \pm 0.007$																	

E. Inheritance of the Diverse Fission Rates

In order to determine whether the diverse fission rates observed are hereditary, the number of fissions which occurred in each line during the first ten days after the ex-conjugants began to divide was correlated with the number of fissions during the following ten days. These coefficients of correlation were obtained (a) for all of the non-conjugant lines which lived from August 6th-26th, (b) for all of the

TABLE V

[illegible][illegible]

For all the ex-conjugant lines, each taken separately (Table VI), there is a well-defined positive correlation of $+0.327 \pm 0.035$. Thus many of the diversities between these are inherited. When, however, the means of the separate tetrads are taken, and the fissions for the first ten days are tabulated against those of the second ten days (Table VII), the correlation rises to $+0.651 \pm 0.039$. In such a table, the process of averaging the different quadrants of the tetrad smoothes out in large measure the accidental differences between the diverse lines, leaving mainly the intrinsic differences, which are inherited; hence the high coefficient of correlation.

TABLE VII

Paramecium aurelia. Means of tetrads. Correlation of number of fissions, August 7-16 and August 17-26.

	6.00	11.00	12.00	12.50	12.67	12.75	13.67	14.00	14.25	14.67	14.75	15.00	15.25	15.33	15.50	15.67	15.75	16.00	16.25	16.33	16.50	16.67	16.75	17.00	17.25	17.33	17.50	
10.50		1																										1
11.00	1																											1
12.00						1																						1
13.50			1																									1
14.00							1 1					1							1	2								6
14.25								1																				1
14.33													1															1
14.50									1					1														2
14.67			1																1									2
14.75												1																1
15.00			1						1			2	1					2			1							8
15.25												1 1		1														3
15.33							1					1		1														3
15.50												1 1	1							1						1		5
15.67				1		2												1	1									5
15.75							1					1					1				1					1		4
16.00								1	1 2				1 2				1	5			1		2					14
16.25								1				1 1						1			1							5
16.33																						1						2
16.50													2	2	1			1	1		1		1					7
16.67								1		1								1 1	1		1							5
16.75																1							1					2
17.00									1										2	2		2		1				6
17.25																	1		2	1								4
17.33														1				1 1										3
17.50													1								1			1				3
17.67																			1						1			3
																												3
	1	1	2	1	1	1	1	7	1	2	1	2	11	5	3	6	3	6	16	4	10	3	2	5	1	1	2	99
	$r = +0.651 \pm 0.039$																											

Thus in a non-conjugant population there is no indication of hereditary diversities between the different lines, while among the lines de-

rived from different ex-conjugants, hereditary diversities are clearly present. A population of ex-conjugant lines consists of diverse biotypes produced by conjugation from a homogeneous clone.

F. Similarity Between the Lines Derived from the Two Members of a Pair of Conjugants

Jennings (1913) and Jennings and Lashley (1913) found that the lines derived from the two members of a pair of conjugants resembled each other more than do the progeny of ex-conjugants derived from different pairs. An attempt was made to determine whether this relation holds for the population studied in this investigation. This was done by correlating the mean number of fissions for twenty days, and the mean average daily fission rate of all lines which lived for more than ten days, of the two tetrads derived from each pair of conjugants. The coefficients of correlation which were obtained were $+0.102 \pm 0.073$ and -0.188 ± 0.070 . Because of the small number of pairs involved and the large probable error obtained, these coefficients of correlation are of uncertain significance. Another experiment using a large number of pairs is planned, in order to investigate this matter further.

SUMMARY AND DISCUSSION

This paper gives an account of an investigation designed to test critically the question whether conjugation produces inherited variation within a clone of *Paramecium aurelia*. An elaborate technique was devised and carried through, to prevent the occurrence of environmental diversities among the lines of descent tested: a synthetic culture fluid was employed; pure cultures of food organisms used, and the glassware standardized to the highest possible degree.

Cultivated in this way, ex-conjugant lines of descent were compared with non-conjugant lines from the same parent clone, with respect to the rates of fission. The results are:

(1) Conjugation greatly increased the variation in fission rate. The population composed of lines descended from ex-conjugants showed a much greater range of variation and a much greater coefficient of variation than did the population derived from non-conjugants. The variation was extended by conjugation mainly in the direction of lowered fission rate. A considerable proportion of the ex-conjugant lines had a lower daily fission rate than any of the non-conjugant lines. Others had as high a fission rate as the non-conjugants (see Table I).

(2) The four quadrants derived from the first two divisions of single ex-conjugants showed when compared only such variation as is

found in non-conjugants; not at all such extreme variations as are found between lines derived from diverse ex-conjugants. The four quadrants from a single ex-conjugant are highly correlated in their fission rates, showing a correlation coefficient of 0.854 ± 0.007 . Such quadrants derived from a single ex-conjugant are thus much more alike in their fission rates than are lines derived from diverse ex-conjugants. There is no indication that the first two fissions occurring after conjugation have any effect in segregating diverse lines, or that they differ in their effects from any other fissions.

(3) The diverse fission rates of lines or populations derived from different ex-conjugants are in large measure inherited, while the differing rates of non-conjugant lines are not inherited.

The work therefore leads to the following conclusions: Conjugation within a clone of *Paramecium aurelia* produces diverse biotypes, having different inherited fission rates. The fissions of a single ex-conjugant do not give origin to diverse biotypes; this is as true of the first two fissions after conjugation as of later fissions.

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VITA

Daniel Raffel was born at Baltimore, Maryland, on August 7, 1899. After receiving his elementary education in the public schools of Baltimore, he attended the Baltimore Polytechnic Institute, from which he was graduated in 1917. He entered the Johns Hopkins University in 1922 and received the degree of Bachelor of Arts from that institution in 1925. The summers of 1924 and 1925 were spent at the Marine Biological Laboratory, Woods Hole, Mass. The same year he was admitted to the Graduate School of the same university. Since 1925 he has been pursuing graduate studies in the field of Zoölogy. He married Rose Mahr in June, 1926. As a fellow of the American Scandinavian Foundation he studied in the Physiological Institute, University of Copenhagen, Copenhagen, Denmark, during the academic year 1926-1927. There he worked with Professor V. Henriques and published a paper on "A micro-method for the quantitative determination of carbon dioxide in the blood and other solutions, and some observations on the efficiency of paraffin oil as a means of keeping carbon dioxide in solution." He was appointed to the American Association for the Advancement of Science table at the Zoölogical Station at Naples where he worked from September, 1927, to January, 1928. Since April, 1928, he has been conducting an investigation in the Zoölogical Laboratory of the Johns Hopkins University. At this University he has been awarded Hopkins Scholarships for the years 1925-1926, 1928-1929, 1929-1930.

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